ABSTRACT

Sequence Analysis of Ribosomal DNA Amplification Products
and Investigations on Protoplast Isolation from
Vesicular-Arbuscular Mycorrhizal Fungi
of the Genus *Gigaspora*

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Research presented in this thesis was aimed at generation of protoplasts from vesicular-arbuscular (VA) mycorrhizal fungi and development of species-specific PCR primers for identification of *Gigaspora margarita* (Glomales, Zygomycetes). Production of axenic extraradical hyphae of VA mycorrhizal fungi for experiments on protoplasts was investigated in two systems: as regrowth hyphae from roots extracted from pot cultures of VA mycorrhizas and as extraradical hyphae from mycorrhizas formed *in vitro*. Surface decontaminated roots extracted from pot cultures did not produce

useful regrowth hyphae. In axenic cultures with excised roots or *Agrobacterium rhizogenes*-transformed roots, hyphae of germinated spores proliferated over and around roots without formation of mycorrhizas.

In the absence of axenic extraradical hyphae, germinated spores and their hyphae were used in experiments on protoplast production. These hyphae proved to be resistant to the action of many commercial lytic enzymes used at high concentrations and in several combinations. The hyphae were not digested by extracellular extracts from cultures of *Bacillus pumilus* or *Streptomyces* no. 6 used alone, or with commercial lytic enzymes, while hyphae of *Rhizopus stolonifer* (Mucorales, Zygomycetes) were readily protoplasted in enzyme mixtures containing *Streptomyces* culture extract.

A method was developed for isolation and purification of high molecular weight DNA from spores of *G. margarita*. Further purification of extracted nucleic acids yielded DNA from which the 5.8S rRNA genes and flanking internal transcribed spacers were amplified by the polymerase chain reaction. Sequences of the 5.8S rRNA gene and internal transcribed spacers of *G. margarita* were determined from amplified fragments cloned into a plasmid. The sequence was aligned with homologous fungal sequences and two PCR primers were derived based on unique sequences in the internal transcribed spacers of *G. margarita*. These primers when tested with plasmids containing rDNA sequences from *G. margarita*, produced amplicons of expected sizes.